

1. **5,336,611**, Aug. 9, 1994, PB92 serine protease muteins and their use in detergents; Christiaan A. G. van Eekelen, et al., 435/221; 510/306, 320, 321, 392, 393, 530 [IMAGE AVAILABLE]

US PAT NO: **5,336,611** [IMAGE AVAILABLE]
PRIM-EXMR: Douglas W. Robinson
ASST-EXMR: Jon P. Weber

L2: 1 of 1

CLAIMS:

CLMS(1)

We claim:

1. A substantially pure serine protease mutein comprising:
a substitution of an amino acid residue at a selected site corresponding to a residue position selected from the group consisting of 116, 126, 127, 128, 160, and 216 of mature PB92 protease obtained from *Bacillus novo* species PB92, wherein said mature PB92 protease has an amino acid sequence as shown in FIG. 4; and wherein
relative to said mature PB92 protease prior to said substitution, the mutein has demonstrated (a) improved wash performance or (b) retained wash performance and improved stability against oxidizing agents.

CLMS(2)

2. A substantially pure serine protease mutein comprising a mutation selected from the group consisting of (M216S); (M216Q); (S160D, M216Q); (S160D, (M216S); (G116V, S126V, P127E, S128K); (G116V, S126G, P127Q, S128I); (G116V, S126L, P127N, S128V); (G116V, S126L, P127Q, S128A); (G116V, S126V, P127M); (G116V, S126H, P127Y); (G116V, S126R, P127S, S128P); (G116V, S126F, P127Q); (G116V, S126F, P127L, S128T); and (S126M, P127A, S128G) of mature PB92 protease obtained from *Bacillus novo* species PB92, wherein said mature PB92 protease has an amino acid sequence as shown in FIG. 4 and wherein relative to said mature PB92 protease prior to said substitution, the mutein has demonstrated (a) improved wash performance or (b) retained wash performance and improved stability against oxidizing agents.

CLMS(3)

3. A substantially pure serine protease mutein comprising one set of mutations selected from the group consisting of:

- (a) G at position 116 to V;
- (b) S at position 126 to F, H, L, M, Q, R, or V;
- (c) P at position 127 to A, E, L, M, N, Q, S, or Y;
- (d) S at position 128 to A, G, K, P, T, or V;
- (e) S at position 160 to C, D, G, or N; and
- (f) M at position 216 to Q or S of mature PB92 protease obtained from *Bacillus novo* species PB92, wherein said mature PB92 protease has an amino acid sequence as shown in FIG. 4 and wherein relative to said mature PB92 protease prior to said substitution, the mutein has demonstrated (a) improved wash performance or (b) retained wash performance and improved stability against oxidizing agents.

CLMS(4)

4. The serine protease mutein according to claim 1, wherein said substitution is at amino acid 216 and is from methionine to serine.

CLMS (5)

5. The serine protease mutein according to claim 1, wherein said substitution is at amino acid 116 and is from glycine to valine.

CLMS (6)

6. The serine protease mutein according to claim 2, wherein said substitution is one set selected from the group consisting of: (G116V, S126V, P127E, S128K); (G116V, S126G, P127Q, S128I); (G116V, S126L, P127N, S128V); (G116V, S126L, P127Q, S128A); (G116V, S126R, P127S, S128P); and (G116V, S126F, P127L, S128T).

CLMS (7)

7. A serine protease mutein according to claim 1, produced by expression of a DNA sequence encoding said proteolytic enzyme in a prokaryotic host strain transformed with an expression vector comprising said DNA sequence.

CLMS (8)

8. A substantially pure serine protease mutein comprising:
a substitution of one or more of amino acid residues at a selected site corresponding to a residue position selected from the group of consisting of 126, 127 and 128 of mature PB92 protease obtained from *Bacillus novo* species PB92, wherein said mature PB92 protease has an amino acid sequence as shown in FIG. 4; and wherein relative to said mature PB92 protease prior to said substitution, the mutein has demonstrated (a) improved wash performance or (b) retained wash performance and improved stability against oxidizing agents.

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L1 1 S 5324653/PN

L2 1 S 5336611/PN

=> d 11 cit,xp,xa,clm

1. **5,324,653**, Jun. 28, 1994, Recombinant genetic means for the production of serine protease muteins; Christiaan A. G. Van Eekelen, et al., 435/221, 172.3, 220, 320.1; 536/23.2 [IMAGE AVAILABLE]

US PAT NO: **5,324,653** [IMAGE AVAILABLE]

L1: 1 of 1

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ASST-EXMR: William W. Moore

CLAIMS:

CLMS (1)

What is claimed is:

1. A method for obtaining a serine protease mutein having at least one improved property relative to a corresponding wild-type protease for use in detergents which comprises the steps of:
mutagenizing a cloned gene encoding a mature PB92 protease obtained from

Bacillus novo species PB92, where said mature PB92 protease has an amino acid sequence as shown in FIG. 4; introducing said gene into a host strain whereby a transformed host strain is obtained; growing said transformed host whereby said mutant gene is expressed and a serine protease mutein differing from said wild-type protease by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 116, 126, 127, 128, 160, 166, 169, and 216, is identified by recovering said mutein and screening it for at least one improved property selected from the group consisting of:

(1) wash performance, and
(2) retained wash performance and improved stability against oxidizing agents in detergents.

CLMS (2)

2. The method according to claim 1, wherein said residue position is 216.

CLMS (3)

3. The method according to claim 2, wherein said amino acid substitution is to a serine.

CLMS (4)

4. An isolated DNA segment consisting essentially of a region encoding a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 116, 126, 127, 128, 160, 166, 169, and 216 from a wild-type PB92 protease obtained from Bacillus novo species PB92, and having, relative to said wild-type PB92 protease, at least one improved property selected from the group consisting of:

(1) wash performance, and
(2) retained wash performance and improved stability against oxidizing agents in detergents.

CLMS (5)

5. An isolated DNA segment consisting essentially of a region encoding a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 87, 116, 126, 127, 128, 160, 166, 169, 212 and 216, wherein said mutein has a substitution at amino acid 116 from glycine to a higher molecular weight non-polar aliphatic amino acid as compared to a wild-type PB92 protease obtained from Bacillus novo species PB92, and having, relative to said wild-type PB92 protease, at least one improved property selected from the group consisting of:

(1) wash performance, and
(2) retained wash performance and improved stability against oxidizing agents in detergents.

CLMS (6)

6. An isolated DNA segment consisting essentially of a region encoding a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected

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from the group consisting of 87, 116, 126, 127, 128, 160, 166, 169, 212, and 216, wherein said mutein has a substitution at amino acid 116 from glycine to valine, isoleucine or leucine as compared to a wild-type PB92 protease obtained from *Bacillus novo* species PB92, and having, relative to said wild-type PB92 protease, at least one improved property selected from the group consisting of:

- (1) wash performance, and
- (2) retained wash performance and improved stability against oxidizing agents in detergents.

CLMS (7)

7. An isolated DNA segment consisting essentially of a region encoding a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 87, 116, 126, 127, 128, 160, 166, 169, 212, and 216, wherein said mutein has a substitution at amino acid 160 to glycine or an anionic amino acid as compared to a wild-type PB92 protease obtained from *Bacillus novo* species PB92, and having, relative to said wild-type PB92 protease, at least one improved property selected from the group consisting of:

- (1) wash performance, and
- (2) retained wash performance and improved stability against oxidizing agents in detergents.

CLMS (8)

8. An isolated DNA segment consisting essentially of a region encoding a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 87, 116, 126, 127, 128, 160, 166, 169, 212, and 216 wherein said mutein has a substitution at amino acid 166 to an anionic amino acid as compared to a wild-type PB92 protease obtained from *Bacillus novo* species PB92, and having, relative to said wild-type PB92 protease, at least one improved property selected from the group consisting of:

- (1) wash performance, and
- (2) retained wash performance and improved stability against oxidizing agents in detergents.

CLMS (9)

9. An isolated DNA segment consisting essentially of a region encoding a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 87, 116, 126, 127, 128, 160, 166, 169, 212, and 216, wherein said mutein has a substitution at amino acid 169 to a non-polar aliphatic amino acid as compared to a wild-type PB92 protease obtained from *Bacillus novo* species PB92, and having, relative to said wild-type PB92 protease, at least one improved property selected from the group consisting of:

- (1) wash performance, and
- (2) retained wash performance and improved stability against oxidizing agents in detergents.

CLMS (10)

10. An isolated DNA segment consisting essentially of a region encoding

a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 116, 126, 127, 128, 160, 166, 169, and 216 wherein said mutein has a substitution from a neutral amino acid to an anionic amino acid as compared to a wild-type PB92 protease obtained from *Bacillus novo* species PB92, and having, relative to said wild-type PB92 protease, at least one improved property selected from the group consisting of:

- (1) wash performance, and
- (2) retained wash performance and improved stability against oxidizing agents in detergents.

CLMS(11)

11. An isolated DNA segment consisting essentially of a region encoding a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 126, 127, and 128 as compared to a wild-type PB92 protease obtained from *Bacillus novo* species PB92, and having, relative to said wild-type PB92 protease, at least one improved property selected from the group consisting of:

- (1) wash performance, and
- (2) retained wash performance and improved stability against oxidizing agents in detergents

CLMS(12)

12. The isolated DNA segment according to claim 11, wherein said mutations are at amino acid 126, from serine to any other non-hydroxylated amino acid; at amino acid 127 to any other acid; and at amino acid 128 to any other amino acid.

CLMS(13)

13. An isolated DNA segment consisting essentially of a region encoding a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 87, 116, 126, 127, 128, 160, 166, 169, 212, and 216, wherein said mutein has a substitution at amino acid 216 from methionine to an amino acid other than a sulfur containing amino acid.

CLMS(14)

14. The isolated DNA segment according to claim 13, wherein said amino acid other than a sulfur containing amino acid is a serine.

CLMS(15)

15. An isolated DNA segment consisting essentially of a region encoding a serine protease mutein characterized as differing by no more than three amino acid substitutions corresponding to a residue position selected from the group consisting of 87, 116, 126, 127, 128, 160, 166, 169, 212 and 216 wherein said serine protease mutein has an amino acid sequence as shown in FIG. 4 except that said amino acid sequence comprises a mutation at least of amino acid 128 as compared to a wild-type PB92 protease obtained from *Bacillus novo* species PB92, and having, relative to said wild-type PB92 protease, at least one improved property selected from the group consisting of:

- (1) wash performance, and
- (2) retained wash performance and improve stability against oxidizing agents in detergents.

CLMS (16)

16. An isolated gene encoding a serine protease mutein which as an amino acid sequence as shown in FIG. 4 except that said amino acid sequence has a mutation selected from the group consisting of (M216S); (M216Q); (S160D); (S160G, N212D); (S160D, M216Q); (S160D, M216S); and (A166D, M169I).

CLMS (17)

17. An isolated DNA segment encoding a serine protease mutein, wherein said mutein has an amino acid sequence as shown in FIG. 4 except that said amino acid sequence has a mutation selected from the group consisting of:

- (a) S at position 160 to C, D, G, or N;
- (b) A at position 166 to D;
- (c) M at position 169 to I;
- (d) M at position 216 to Q or S; and.

CLMS (18)

18. An isolated DNA segment encoding a mutant proteolytic enzyme wherein said enzyme has an amino acid sequence as shown in FIG. 4 except that said amino acid sequence has a mutation selected from the group consisting of: (G116V, S126V, P127E, S128K); (G116V, S126G, P127Q, S128I); (G116V, S126L, P127N, S128V); (G116V, S126L, P127Q, S128A); (G116V, S126R, P127S, S128P); (G116V, S126F, P127L, S128T); (S126M, P127A, S128G); (S126M, P127A, S128G, S160D); and (G116V, S126N, P127S, S128A, S160D).

CLMS (19)

19. An isolated DNA segment encoding a serine protease mutein, wherein said mutein has an amino acid sequence as shown in FIG. 4 except that said amino acid sequence has a mutation selected from the group consisting of:

- (a) G at position 116 to V;
- (b) S at position 126 to F, H, L, M, Q, R, or V;
- (c) P at position 127 to A, E, L, M, N, Q, R, or Y;
- (d) S at position 128 to A, G, K, P, T, or V.

CLMS (20)

20. An isolated gene encoding a serine protease mutein which has an amino acid sequence as shown in FIG. 4 except that said amino acid sequence has a mutation selected from the group consisting of (G116V, S126V, P127E, S128K); (G116V, S126G, P127Q, S128I); (G116V, S126L, P127N, S128V); (G116V, S126L, P127Q, S128A); (G116V, S126V, P127M); (G116V, S126H, P127Y); (G116V, S126R, P127S, S128P); (G116V, S126F, P127Q); (G116V, S126F, P127L, S128T); (S126M, P127A, S128G); and (S126M, P127A, S128G, S160D).

CLMS (21)

21. An isolated gene encoding a serine protease mutein which has an amino acid sequence as shown in FIG. 4 except that said amino acid sequence has a mutation (G116V, S126N, P127S, S128A, S160D).

CLMS (22)

22. The isolated DNA segment according to claim 4, wherein said DNA segment is further included on a vector, said vector comprising sequences for the expression of said DNA segment.

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